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**Effects of yellow mealworm larvae (*Tenebrio molitor*)
inclusion in diets for female broiler chickens: implications
for animal health and gut histology**

I. Biasato^a, L. Gasco^{b,c}, M. De Marco^a, M. Renna^b, L. Rotolo^b,
S. Dabbou^b, M.T. Capucchio^a, E. Biasibetti^a, M. Tarantola^{a,d}, C.
Bianchi^a, L. Cavallarin^c, F. Gai^c, L. Pozzo^{c,e}, D. Dezzutto^f, S.
Bergagna^f, L., and A. Schiavone^{a,d}

^aDepartment of Veterinary Sciences, University of Turin, Largo
Paolo Braccini 2, 10095 Grugliasco (TO), Italy

^bDepartment of Agricultural, Forest and Food Sciences,
University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco
(TO), Italy

^cInstitute of Science of Food Production, National Research
Council, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

^dInstitute of Multidisciplinary Research on Sustainability,
University of Turin, Via Accademia Albertina 13, 10100,
Turin, Italy.

^eInstitute of Biology and Agricultural Biotechnology, National
Research Council, Via Moruzzi 1, 56124, Pisa, Italy.

^fVeterinary Medical Research Institute for Piemonte, Liguria
and Valle d'Aosta, Via Bologna 148, 10154, Turin, Italy.

25 *Corresponding Author: Prof. Laura Gasco, Department of
26 Agricultural, Forest and Food Sciences, University of Turin,
27 Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy. Tel:
28 +39-011-6708574; Fax: +39-011-6708563; E-mail:
29 laura.gasco@unito.it

30

31 E-mail addresses:

32 IB: ilaria.biasato@unito.it

33 LG: laura.gasco@unito.it

34 MDM: michele.demarco.to@gmail.com

35 LR: lucarotolo@gmail.com

36 MR: manuela.renna@unito.it

37 SD: sihem.dabbou@yahoo.fr

38 MTC: mariateresa.capucchio@unito.it

39 EB: elena.biasibetti@unito.it

40 MT: martina.tarantola@unito.it

41 CB: chiara.bianchi@unito.it

42 LC: laura.cavallarin@ispa.cnr.it

43 FG: Francesco.gai@ispa.cnr.it

44 LP: luisa.pozzo@ibba.cnr.it

45 DD: daniela.dezzutto@izsto.it

46 SB: stefania.bergagna@izsto.it

47 AS: achille.schiavone@unito.it

48

49 **Abstract**

50 The aim of the present study was to evaluate the animal
51 performance, haematochemical parameters, intestinal
52 morphology and histological features of broiler chickens fed
53 diets including *Tenebrio molitor* (TM) larvae meal. A total of
54 160 female broiler chicks (Ross 708) at one-day of age were
55 randomly allotted to four dietary treatments: a control (C)
56 group and three TM groups, in which TM meal was included at
57 50, 100 and 150 g/kg, respectively. Each group consisted of
58 five pens as replicates, with eight chicks per pen. After the
59 evaluation of growth performance and haematochemical
60 parameters, two birds per pen were slaughtered at 40 days and
61 carcass traits were recorded. Morphometric investigations were
62 performed on duodenum, jejunum and ileum and
63 histopathological alterations were assessed for liver, spleen,
64 thymus, bursa of Fabricius, kidney and heart. The live weight
65 (LW) showed a linear (12 days, $P < 0.05$, maximum with
66 TM15) and quadratic response (40 days, $P < 0.05$, maximum
67 with TM5) to dietary TM meal inclusion. The average daily
68 gain (ADG) showed a linear increase (1-12 days, $P < 0.05$,
69 maximum with TM15) in response to TM meal utilization. A
70 linear effect (1-12 and 12-25 days, $P < 0.01$ and $P < 0.05$,
71 maximum with TM15 and TM5) was observed for the daily
72 feed intake (DFI). The feed conversion ratio (FCR) showed a
73 linear response to TM utilization in the period 12-25 days ($P <$

0.01, maximum with TM15). A quadratic effect ($P < 0.05$, maximum with TM5) was observed for the carcass weight. The abdominal fat weight and percentage showed a linear response to dietary TM meal inclusion ($P < 0.05$ and $P < 0.01$, maximum with TM15 and TM10). A quadratic increase ($P < 0.05$, maximum with TM10) was observed for the erythrocytes, while the albumin and GGT showed a linear and quadratic decrease ($P < 0.05$, minimum with TM10) in relation to TM utilization. Gut morphology and histopathological findings were not significantly influenced ($P > 0.05$) by dietary TM meal inclusion. The present study suggests that increasing levels of dietary TM meal inclusion in female broiler chickens diets may improve body weight and feed intake, but can partially worsen feed efficiency. However, positive effects on carcass traits and haematochemical parameters related to TM meal utilization are observed, along with no negative influence on gut morphology and histological findings.

91

92 **Keywords**

93 Poultry; *Tenebrio molitor*; insect meal; growth performance;
94 histology; morphometry.

95

96 **Introduction**

97 World population is expected to increase by over a third,
98 reaching over 9 billion people in 2050. This trend suggests that
99 market demand for food will continue to grow. In particular,
100 the demand for cereals and protein sources in both human food
101 and animal feed is projected to have an exponential growth by
102 2050 (FAO, 2013). Consequently, the world supply of some
103 conventional feedstuffs like soybean and maize will
104 increasingly compete between humans and livestock.
105 Therefore, the foremost gamble will be the identification of
106 alternative sources of protein, energy and other nutrients for
107 livestock, in order to avoid such a competition.

108 The potential of insects for becoming a standard ingredient in
109 animal feeds has already been emphasized by several studies
110 (Veldkamp et al., 2012; Van Huis, 2013; Henry et al., 2015),
111 because of the high quality and quantity of protein (Makkar et
112 al., 2014), the low competitiveness with human food (Ballitoc
113 and Sun, 2013) and the reduction of the environmental impact
114 (Oonincx and de Boer, 2012; Makkar et al., 2014; Sánchez-
115 Muros et al., 2014). Currently, the considered most valuable
116 insect species to be used in livestock feeds are *Hermetia*
117 *illucens* L. (black soldier fly), *Musca domestica* L. (common
118 house fly), *Tenebrio molitor* L. (yellow mealworm), *Bombyx*
119 *mori* L. (silkworm) and several grasshoppers (Van Huis, 2013).
120 In particular, *Tenebrio molitor* (TM) and *Hermetia illucens*

121 have recently been used in poultry (Biasato et al., 2016; Bovera
122 et al., 2016; Schiavone et al., 2017a) and fish (Belforti et al.,
123 2015; Gasco et al., 2016; Renna et al., 2017) feeding. Yellow
124 mealworms are already industrially produced as feed for pets
125 and zoo animals, such as birds, reptiles, small mammals,
126 amphibians and fish, and TM larvae are easily bred on dried
127 waste materials, being able to recycle them into high-quality
128 feed with less energy cost, land area utilization and footprints
129 (Makkar et al., 2014). The influence of TM-based diets on
130 growth performance (Bovera et al., 2015; Bovera et al., 2016;
131 Biasato et al., 2016) haematochemical profile (Bovera et al.,
132 2015; Biasato et al., 2016) and carcass traits (Ballitoc and Sun,
133 2013; Bovera et al., 2016; Bisato et al., 2016), has recently
134 been investigated. Gut morphology, which has been reported to
135 be widely affected by dietary modifications in broilers
136 (Laudadio et al., 2012; Gopinger et al., 2014; Qaisrani et al.,
137 2014), has also been evaluated in free-range chickens fed diets
138 with dietary TM larvae meal inclusion (Biasato et al., 2016).
139 Despite insect meals being considered suitable ingredients for
140 poultry feeding (Veldkamp et al., 2012; van Huis, 2013;
141 Makkar et al., 2014), the implications of their utilization on
142 poultry health and gut development are still very limited. The
143 aim of the present study was to evaluate the growth
144 performance, haematochemical parameters, carcass traits,

145 intestinal morphology and histological features of female
146 broiler chickens fed diets including TM meal.

147

148 **Materials and Methods**

149 ***Birds and Husbandry***

150 The present trial was performed in collaboration with a local
151 poultry corporation named “O.R.A. Agricola S.r.l.” sited in
152 Cherasco (Cuneo, Italy). The experimental protocol was
153 designed according to the guidelines of the current European
154 and Italian laws on the care and use of experimental animals
155 (European Directive 86 609/EEC, put into law in Italy with
156 D.L. 116/92). Furthermore, the experimental protocol was
157 approved by the Ethical Committee of the Department of
158 Veterinary Sciences of the University of Turin (Italy). A
159 poultry house of 14 m wide × 141 m long × 4.7 m high,
160 equipped with waterproof floor and wall, completely covered
161 by tiles and provided with automatic ventilation system was
162 used.

163 A total of 160 female broiler chicks (Ross 708) at one-day of
164 age were randomly allotted to 4 dietary treatments, each
165 consisting of 5 pens as replicates with 8 chicks per pen. Each
166 pen was 1.20 m wide × 1.20 m long and was equipped with a
167 feeder occupying a surface of almost 1800 cm², three nipple
168 drinkers and rice hulls as litter. During the first three weeks, the
169 animals were heated by infrared lamps to maintain the suitable

170 temperature according to standard breeding practices (Aviagen,
171 2014). Lighting schedule was 23 hours light : 1 hour darkness
172 until day 3 and 18 h light : 6 hours darkness until slaughter age.
173 At hatching, all chicks received vaccination against Newcastle
174 disease, Gumboro disease, infectious bronchitis and
175 coccidiosis. Vaccine recalls were performed on day 9 for
176 infectious bronchitis and on day 18 for Gumboro and
177 Newcastle diseases.

178

179 *Diets*

180 A basal diet based on corn meal, corn gluten meal and soybean
181 meal was formulated and served as control (C) group, while 50,
182 100 and 150 g/kg full-fat TM larvae meal (Gaobeidian
183 Shannong Biology CO., LTD, Gaobeidian, Hebei province,
184 China) inclusion as a partial replacement of soybean meal, corn
185 gluten meal and soybean oil constituted the three experimental
186 treatment groups (TM5, TM10 and TM15) (Table 1). TM meal
187 nutritive composition and energy content were the following:
188 dry matter, 939.0 (g/kg as fed); organic matter, 912.0 (g/kg as
189 fed); crude protein (CP), 519.0 (g/kg as fed); ether extract (EE),
190 236.0 (g/kg as fed); 117.0 (g/kg as fed); neutral detergent fibre
191 (NDF); 79.5 (g/kg as fed); acid detergent fibre (ADF); DL-
192 methionine, 10.1 (g/kg as fed); L-lysine, 35.9 (g/kg as fed);
193 gross energy, 24.4; apparent metabolizable energy (AMEn),
194 16.02 (MJ/kg DM). Three different diets were used per each

195 dietary treatments during the three phases of growth: a starter
196 diet (days 1 to 12), a grower diet (days 12 to 25) and a finisher
197 diet (day 25 to 40). For each phase, the experimental diets were
198 isonitrogenous and isoenergetic and were formulated using the
199 AMEn values for TM measured *in vivo* for broiler chickens (De
200 Marco et al., 2015). Diets met or exceeded NRC (1994)
201 requirements and were adjusted according to Aviagen (2014)
202 broiler nutrition specifications. Feed and water were provided
203 *ad libitum*.

204

205 ***Chemical analysis***

206 The diets were ground to pass through a 0.5-mm sieve and
207 stored in airtight plastic containers for DM (method number
208 943.01), ash (method number 924.05), CP (method number
209 954.01), EE (method number 920.39), NDF (method number
210 2002.04) and ADF (method number 973.18) determination
211 (AOAC, 2004).

212

213 ***Growth Performances***

214 Health status and mortality were daily monitored during the
215 whole experimental period. Live weight (LW) of the animals
216 was recorded at an individual level at the beginning of the trial,
217 at day 12, 25 and 40. Average daily gain (ADG) and average
218 daily feed intake (DFI) were recorded at an individual and a
219 pen level, respectively, at the end of each growth period. Feed

220 conversion ratio (FCR) was determined for each growth period
221 and for the overall experimental period. All measurements were
222 made on the pen basis using a high precision electronic scale
223 (Sartorius – Signum®).

224

225 *Slaughtering procedure and recordings*

226 At day 40, all birds were individually weighed and 10
227 broilers/diet (2 birds/pen) were chosen on the basis of pen
228 average LW and identified with a shank ring.

229 All selected animals were slaughtered in a commercial abattoir.

230 The plucked and eviscerated carcasses were obtained and the

231 head, neck, feet and abdominal fat were removed to obtain

232 carcass-for-grilling. The weights of liver, spleen, gizzard and

233 abdominal fat were immediately recorded. All slaughtered

234 carcasses were stocked in a cooling chamber (0-4 °C) for 24 h.

235 Weights of carcass-for-grilling, breast and thighs were

236 successively recorded. Carcass-for-grilling, breast, thigh and

237 organs weights were also expressed as percentage of LW.

238 Collected feet were examined macroscopically using the

239 Swedish FPD scoring system (Ekstrand et al., 1997). According

240 to this system, 0 = no lesion, slight discoloration of the skin or

241 healed lesion; 1= mild lesion, superficial discoloration of the

242 skin and hyperkeratosis; 2 = severe lesion, affected epidermis,

243 blood scabs, hemorrhage and severe swelling of the skin.

244

245 ***Haematological and serum parameters***

246 At slaughter, blood samples were collected from the identified
247 broilers: 2.5 mL was placed in an EDTA tube and 2.5 mL in a
248 serum-separating tube. A blood smear was prepared, using one
249 glass slide for each bird, from a drop of blood without
250 anticoagulant. The smears were stained using May-Grünwald
251 and Giemsa stains (Campbell, 1995). The total red and white
252 blood cell counts were determined in an improved Neubauer
253 haemocytometer on blood samples previously treated with a
254 1:200 Natt-Herrick solution. One hundred leukocytes, including
255 granular (heterophils, eosinophils and basophils) and non-
256 granular (lymphocytes and monocytes) leukocytes, were
257 counted on the slide and the heterophils to lymphocytes (H/L)
258 ratio was calculated. The tubes without anticoagulant were left
259 to clot in a standing position at room temperature for
260 approximately two hours to obtain serum. The serum was
261 separated by means of centrifugation at $700 \times g$ for 15 minutes
262 and frozen at -80°C until analysis. The total proteins were
263 quantified by means of the “biuret method” (Bio Group
264 Medical System kit; Bio Group Medical System, Talamello
265 (RN), Italy); the electrophoretic pattern of the serum was
266 obtained using a semi-automated agarose gel electrophoresis
267 system (Sebia Hydrasys®, Norcross, GA, USA). The alanino-
268 aminotransferase (ALT), aspartate-aminotransferase (AST),
269 gamma glutamyl transferase (GGT), triglycerides, cholesterol,

270 glucose, phosphorus, magnesium, iron, uric acid and creatinine
271 serum concentrations were measured by means of enzymatic
272 methods in a clinical chemistry analyzer (Screen Master Touch,
273 Hospitex diagnostics Srl., Firenze, Italy).

274

275 *Histomorphological investigations*

276 The slaughtered animals were submitted to
277 anatomopathological investigations. Intestinal segment samples
278 (approximately 5 cm in length) of duodenum, jejunum, ileum
279 and caecum were excised from each bird and flushed with 0.9%
280 saline to remove all the content. The collected segments of
281 intestine were the loop of the duodenum, the tract before
282 Meckel's diverticulum (jejunum), the tract before the ileocolic
283 junction (ileum) and the apex of the caeca (caecum). Gut
284 segments were fixed in Carnoy's solutions for morphometric
285 analysis. Tissues were routinely embedded in paraffin wax
286 blocks, sectioned at 5 μ m thickness, mounted on glass slides
287 and stained with Haematoxylin & Eosin (HE). The evaluated
288 morphometric indices were Vh (from the tip of the villus to the
289 crypt), Cd (from the base of the villus to the submucosa) and
290 the Vh/Cd ratio (Laudadio et al., 2012). Morphometric analyses
291 were performed on 10 well-oriented and intact villi and 10
292 crypts chosen from duodenum, jejunum and ileum (Qaisrani et
293 al., 2014). Samples of liver, spleen, thymus, bursa of Fabricius,
294 kidney and heart were also collected from each animal and

295 fixed in 10% buffered formalin solution for histopathological
296 examination. Tissues were processed in the same way as gut
297 and the following histopathological alterations were evaluated:
298 white pulp hyperplasia and depletion in spleen, cortical
299 depletion in thymus, follicular depletion and intrafollicular
300 cysts in bursa of Fabricius and lymphoid tissue activation in
301 liver (Biasato et al., 2016). Heart and kidney were assessed for
302 inflammatory and degenerative diseases. The observed
303 histopathological findings were evaluated using a
304 semiquantitative scoring system as previously assessed by
305 Biasato et al. (2016): absent/minimal (score = 0), mild (score =
306 1) and severe (score = 2).

307

308 *Statistical Analysis*

309 IBM SPSS Statistics V20.0.0 software was used to perform
310 statistical analysis. Shapiro-Wilk's test established normality or
311 non-normality of distribution. The experimental unit was the
312 pen for growth performance, haematochemical parameters and
313 carcass traits and bird for histomorphological findings. Data
314 collected for growth performance, blood parameters and
315 carcass traits were tested by one-way ANOVA, evaluating the
316 effect of dietary TM inclusion by polynomial contrasts. χ^2 test
317 was performed to evaluate the association between the
318 mortality rate and the dietary treatments. Intestinal
319 morphometric indices were analyzed by fitting a general linear

320 model (GLM). The GLM allowed the morphometric indices
321 (Vh, Cd and Vh/Cd, separately) to depend on three fixed
322 factors (diet, intestinal segment and interaction between diet
323 and intestinal segment). The interactions between the levels of
324 the fixed factors were evaluated by pairwise comparisons.
325 Statistical analysis was performed by procedure “General
326 Linear Models > Univariate”. Histopathological and FPD
327 scores were analyzed by Kruskal-Wallis test (post-hoc test:
328 Dunn’s Multiple Comparison test). P values < 0.05 were
329 considered statistically significant. The results were expressed
330 as mean and pooled standard error of the mean (SEM).

331

332 **Results**

333 *Growth performance*

334 No clinical signs were observed and the birds remained healthy
335 during the whole experimental period. The mortality rates of C
336 (2.5%), TM5 (2.5%), TM10 (0%) and TM15 (2.5%) groups
337 were not significantly different among treatments ($P > 0.05$).
338 Growth performance of the broiler chickens are summarized in
339 Table 2. At 12 days of age, the LW increased linearly with
340 increasing TM meal levels ($P < 0.05$) and the linear response
341 increased to a maximum corresponding to the inclusion of 150
342 g/kg of TM meal. At 25 days of age, no significant effects
343 related to TM meal utilization were observed. At 40 days of
344 age, the LW showed quadratic response to increasing TM meal

345 levels ($P < 0.05$), with a maximum corresponding to the
346 inclusion of 50 g/kg of TM meal. In the period from 1-12 days
347 of age, the ADG increased linearly with increasing TM meal
348 levels ($P < 0.05$) and the linear response increased to a
349 maximum corresponding to the inclusion of 150 g/kg of TM
350 meal. On the contrary, the ADG showed no differences ($P >$
351 0.05) in the periods from 12 to 25 and 25 to 40 days of age In
352 the period from 1 to 12 days of age, the response of DFI to the
353 effect of TM meal inclusion was statistically significant ($P <$
354 0.01). In particular, the linear response increased to a maximum
355 corresponding to the inclusion of 150 g/kg of TM meal. In the
356 period from 12 to 25 days of age, the DFI increased linearly
357 with increasing TM meal levels ($P < 0.05$) and the linear
358 response increased to a maximum corresponding to the
359 inclusion of 50 g/kg of TM meal. On the contrary, the DFI
360 showed no differences ($P > 0.05$) in the period from 25 to 40
361 days of age. In the periods from 1 to 12, 25 to 40 and 1 to 40
362 days of age, the FCR was similar among the dietary treatments
363 ($P > 0.05$). Differently, the FCR showed linear response to
364 increasing TM meal levels in the period from 12 to 25 ($P <$
365 0.01), with a maximum corresponding to the inclusion of 150
366 g/kg of TM meal.

367

368 *Slaughtering performance and footpad dermatitis (FPD)*

369 *score*

370 Table 3 summarizes the slaughtering performance of the broiler
371 chickens. The carcass weight increased quadratically with
372 increasing TM meal levels ($P < 0.05$) and the quadratic
373 response increased to a maximum corresponding to the
374 inclusion of 50 g/kg of TM meal. The abdominal fat weight
375 showed linear response to increasing TM meal levels ($P <$
376 0.05), with a maximum corresponding to the inclusion of 150
377 g/kg of TM meal. Similarly, the abdominal fat percentage
378 increased linearly with increasing TM meal levels ($P < 0.01$)
379 and the linear response increased to a maximum corresponding
380 to the inclusion of 100 g/kg of TM meal. On the contrary, no
381 significant effects related to TM meal utilization were observed
382 for the other carcass traits ($P > 0.05$). FPD scores (C: 0.40;
383 TM5: 0.20; TM10: 0.20; TM15: 0.00) were also not influenced
384 by dietary TM meal inclusion ($P > 0.05$).

385

386 *Haematological and serum parameters*

387 Haematological and serum biochemical traits of the broiler
388 chickens are summarized in Table 4. The erythrocytes
389 increased quadratically with increasing TM meal levels ($P <$
390 0.05) and the quadratic response increased to a maximum
391 corresponding to the inclusion of 100 g/kg of TM meal. The
392 albumin showed linear response to increasing TM meal levels
393 ($P < 0.05$), with a minimum corresponding to the inclusion of
394 100 g/kg of TM meal. Similarly, the GGT decreased

395 quadratically with increasing TM meal levels ($P < 0.05$) and the
396 quadratic response decreased to a minimum corresponding to
397 the inclusion of 100 g/kg of TM meal. On the contrary, no
398 significant effects related to TM meal utilization were observed
399 for the other blood and serum parameters ($P > 0.05$).

400

401 *Histomorphological investigations*

402 The effects of the diet, intestinal segment and interaction
403 between diet and intestinal segment on gut morphometric
404 indices of the broiler chickens are shown in Tables 5 and 6.
405 There was no influence of diet or interaction between diet and
406 intestinal segment ($P > 0.05$) on the gut morphometric indices.
407 On the contrary, Vh, Cd and Vh/Cd depended on intestinal
408 segment ($P < 0.001$, $P = 0.001$ and $P < 0.001$, respectively)
409 (Table 5). In particular, the duodenum showed higher Vh ($P <$
410 0.001) than the jejunum and ileum. Furthermore, higher Cd (P
411 < 0.001) was found in the duodenum and jejunum than the
412 ileum. Similarly, the duodenum and jejunum showed higher
413 Vh/Cd ($P < 0.001$) than the ileum (Table 6).

414 Histopathological alterations were observed in all the dietary
415 treatments and developed in spleen, thymus, bursa of Fabricius
416 and liver, while heart and kidney showed no significant
417 findings. Spleen ($C = 1.50 \pm 0.22$; TM5 = 1.20 ± 0.25 ; TM10 =
418 1.10 ± 0.31 ; TM15 = 1.10 ± 0.28), thymus ($C = 0.10 \pm 0.10$;
419 TM5 = 0.30 ± 0.15 ; TM10 and TM15 = 0.10 ± 0.10), bursa of

420 Fabricius ($C = 1.90 \pm 0.10$; $TM5 = 1.50 \pm 0.27$; $TM10 = 1.80 \pm$
 421 0.20 ; $TM15 = 1.70 \pm 0.21$) and liver ($C = 0.90 \pm 0.23$; $TM5 =$
 422 1.10 ± 0.23 ; $TM10 = 0.90 \pm 0.23$; $TM15 = 0.30 \pm 0.15$) scores
 423 were not influenced by dietary TM meal inclusion. Spleen
 424 showed moderate ($C = 30\%$ of the broilers; $TM5 = 40\%$; $TM10$
 425 $= 10\%$; $TM15 = 30\%$) to severe ($C = 60\%$; $TM5 = 40\%$; $TM10$
 426 $= 50\%$; $TM15 = 40\%$) white pulp depletion or hyperplasia. The
 427 10% I, 20% (TM5), 40% (TM10) and 30% (TM15) of the
 428 animals had normal spleen (Fig. 1A-B). In thymus, moderate
 429 cortical depletion was found in all the groups ($C = 10\%$ of the
 430 broilers; $TM5 = 30\%$; $TM10$ and $TM15 = 10\%$). A normal
 431 thymus was observed in 90% I, 70% (TM5) and 90% (TM10
 432 and TM15) of the animals. Bursa of Fabricius showed moderate
 433 (C and $TM5 = 10\%$ of the broilers; $TM10 = 0\%$; $TM15 = 10\%$)
 434 to severe ($C = 80\%$; $TM5 = 70\%$; $TM10 = 90\%$; $TM15 = 80\%$)
 435 follicular depletion. The 0% I, 20% (TM5), 10% (TM10) and
 436 10% (TM15) of the animals had normal bursa of Fabricius (Fig.
 437 1C-D). In liver, moderate (C , $TM5$ and $TM10 = 50\%$ of the
 438 broilers; $TM15 = 30\%$) to severe ($C = 20\%$; $TM5 = 30\%$;
 439 $TM10 = 20\%$; $TM15 = 0\%$) perivascular lymphoid tissue
 440 activation. A normal liver was observed in 30% I, 20% (TM5),
 441 30% (TM10) and 70% (TM15) of the animals.

442

443 **Discussion**

444 ***Growth performances***

445 Growth performances of the broiler chickens of the present
446 study were consistent with the reference values recorded in the
447 commercial farm in which the trial was conducted.

448 The body weight, weight gain and feed intake of the birds in
449 the present trial improved with increasing levels of TM meal
450 inclusion, but the feed efficiency resulted partially impaired.

451 Little information on the influence of dietary TM meal
452 inclusion in broiler chickens is currently available. Ramos-
453 Eldoury et al. (2002) and Biasato et al. (2016) did not show any
454 effects for the growth performance in fast-growing and
455 intermediate-growing chickens, respectively, fed diets in which
456 the TM inclusion level ranged from 50 to 100 g/kg. Differently,
457 Ballitoc and Sun (2013) and Bovera et al. (2015) observed
458 improved growth performance in fast-growing chickens fed
459 diets with low (from 5 to 100 g/kg) or high (296 g/kg) TM
460 inclusion levels, respectively. Similar findings were obtained
461 by Loponte et al. (2017) in partridges fed diets in which the TM
462 inclusion level ranged from 250 to 500 g/kg. Recently, Islam
463 and Yang (2017) also found a positive effect of a mealworm-
464 based probiotic on broiler growth performance. Some authors
465 also explored the possibility to use other insect meals in poultry
466 feeding. Adeniji (2007) and Hwangbo et al. (2009) studied the
467 effects of housefly-maggots as feed supplement in the diet of
468 broiler chickens: the first found no differences for growth
469 performance with inclusion levels ranging from 55 to 220 g/kg,

470 while the latter (inclusion rate: 50-200 g/kg) observed a linear
471 increase in LW gain. Ijaiya and Eko (2009) evaluated the
472 effects of replacing dietary fishmeal with silkworm meal
473 (inclusion rate: 22-93 g/kg) on growth performance of broiler
474 chickens, finding no differences related to insect meal
475 utilization. Also Oyegoke et al. (2006) and Wang et al. (2005)
476 observed no adverse effects on growth performance of broiler
477 chickens fed diets with *Cirina forda* (inclusion rate: 20-40
478 g/kg) and *Gryllus testaceus* (inclusion rate: 50-150 k/kg),
479 respectively. Cullere et al. (2016) recently studied the influence
480 of the inclusion (from 100 to 150 g/kg) of *Hermetia illucens*
481 meal in quail diet, finding no differences for growth
482 performances. Schiavone et al. (2017b) also evaluated the
483 effects of replacing soybean oil with *Hermetia illucens* meal
484 (inclusion rate: 500-1000 g/kg) on growth performance of
485 broiler chickens, finding no differences related to insect meal
486 utilization. The wide variability of the results obtained in the
487 previous studies may be related to the nutritive value of the
488 insect meal used, which can be influenced by the species, the
489 insect life stage (adult, larva or pupa) and the insect rearing
490 substrate (Sánchez-Muros et al., 2014).

491 The improvement of feed intake observed in the birds fed TM
492 diets of the present trial was considered suggestive of increased
493 feed palatability in relation to the addition of yellow
494 mealworms, since insects are naturally consumed by wild birds

495 and free-range poultry (Zuidhof et al., 2003). In particular, the
496 increased DFI observed in the starter period, which was
497 accompanied by increased LW and ADG and unaffected FCR,
498 was quite relevant. Indeed, starter period (from hatch to 10
499 days) is considered the most important in broiler production,
500 since growth and development take place at an incredible rate
501 during it. In this period, the chicks' weight quadruples, thus
502 influencing the following growth rate (Aviagen, 2014). On the
503 contrary, the increased DFI observed in the growing period was
504 accompanied by unaffected LW and ADG and subsequently
505 impaired FCR, thus representing a negative effect related to
506 TM meal utilization. De Marco et al. (2015) speculated that the
507 chitin contained in the exoskeleton of the TM meal may
508 negatively influence the apparent digestibility coefficient of the
509 total tract of nutrients. Furthermore, Ravindran and Blair
510 (1993) pointed out that the chitin of insects is difficult to digest
511 by domestic poultry. As suggested by Rumpbold and Schlüter
512 (2013), the partial chitin removal through high pressure
513 processing could improve the use of insects as feeding
514 ingredient thanks to disruption of the link between some chitin-
515 bound proteins. However, the limited number of birds included
516 in the current trial could have influenced the data interpretation.
517 The results obtained need to be confirmed on a larger number
518 of animals.

519

520 *Slaughtering performance and footpad dermatitis (FPD)*
521 *score*

522 The majority of the carcass traits of the broilers in the present
523 trial were not influenced by dietary TM meal inclusion, as
524 previously observed by Bovera et al. (2016) and Biasato et al.
525 (2016). Similar findings were obtained by Cullere et al. (2016)
526 and Schiavone et al. (2017) in broiler quails and chickens fed
527 diets with *Hermetia illucens* meal and fat, respectively.
528 However, the carcass weight, abdominal fat weight and
529 abdominal fat percentage increased with increasing levels of
530 TM meal utilization. Loponte et al. (2017) also observed
531 improved carcass weights when TM and *Hermetia illucens*
532 meals were included in the diets of partridges. As already
533 suggested by them, the differences in the eviscerated carcass
534 weights can be partially explained by the increased final LW of
535 the birds. Similar findings in terms of improved eviscerated
536 carcass weights were obtained by Khatun et al. (2003),
537 Hwangbo et al. (2009) and Ballitoc and Sun (2013), who also
538 observed improved slaughter, dressed carcass, breast muscle
539 and thigh muscle weights and dressing percentage in broilers
540 fed diets with different insect meals inclusion. The differences
541 in the abdominal fat weight and abdominal fat percentage
542 observed in the birds of the present study are also in agreement
543 with Ballitoc and Sun (2013) and suggests that yellow

mealworm utilization may improve fat mass in broiler chickens
(USDA, 2011).

The majority of FPD scores obtained in the present trial was
zero and no differences were found in relation to TM meal
utilization. This is a positive result, since a low prevalence and
severity of FPD is highly desirable as far as health of birds and
product quality are concerned (Meluzzi et al., 2008).

Haematological and serum parameters

All the blood parameters obtained in the present trial fell within
the physiological ranges (Lumej, 2008), thus suggesting that
TM meal utilization does not affect health status of the animals.
In particular the H/L ratio, that is commonly used as indicator
of stress in poultry (De Marco et al., 2013; Salamano et al.,
2010), was not affected by dietary TM meal inclusion. As
already observed by Bovera et al. (2015) and Biasato et al.
(2016), the majority of the haematochemical and serum
biochemical traits were not affected by yellow mealworm
inclusion in the birds of the present study. Similar findings
were obtained by Schiavone et al. (2017b) in broilers fed diets
with *Hermetia illucens* fat. However, the erythrocytes increased
and albumin and GGT decreased with increasing levels of TM
meal utilization Loponte et al. (2017) also observed lower
albumin when TM and *Hermetia illucens* meals were included
in the diets of partridges. Interestingly, this finding was

569 accompanied by the increase of albumin/globulin ratio, which
570 was also reported by Bovera et al. (2015) and ascribed to the
571 properties of chitin contained in insect meal. High globulin
572 concentrations and low albumin/globulin ratios generally
573 indicate better disease resistance and immune response of birds
574 (Griminger and Scanes, 1986). Another interesting finding is
575 represented by the decrease of GGT serum levels in TM
576 animals. Indeed, a high GGT concentration in birds is used as
577 an indicator of liver disease and bile flow disorders (Ognik and
578 Krauze, 2016). Therefore, GGT reduction can be considered a
579 positive effect related to TM meal utilization.

580

581 *Histomorphological investigations*

582 Dietary TM meal inclusion did not influence the gut
583 morphology of the birds of the present study, as already
584 observed by Biasato et al. (2016). Morphometric measurements
585 of Vh and Cd are generally used to assess intestinal
586 development (Franco et al., 2006), since represent useful
587 indicators of gut proliferative and absorptive compartments
588 (Lenhardt and Mozes, 2003). The Vh/Cd ratio is also evaluated,
589 because it gives an indication of the likely maturity and
590 functional capacity of the enterocytes (Hampson, 1986). As
591 previously reported (Uni et al., 1999; Iji et al., 2001; Biasato et
592 al., 2016), the present study confirms that both duodenum and
593 jejunum show a greater morphological development compared

594 with the ileum. Indeed, the duodenum is the intestinal tract with
595 the fastest cell renewal, and is also the first segment of the
596 small intestine to receive physical, chemical and hormonal
597 stimuli provoked by diet (Macari, 1998). Furthermore, the
598 jejunum is an important site for nutrient digestion (Iji et al.,
599 2001). Therefore, dietary TM inclusion preserves a
600 proximodistal decreasing gradient of the morphometric indexes
601 from the duodenum to the ileum, thus suggesting the
602 maintenance of the physiological gut development.

603 The broiler chickens of the present trial showed different
604 degrees of lymphoid system activation, with no differences
605 related to dietary TM meal inclusion. This result could be
606 related to the stress occurrence in modern poultry rearing
607 operations. Stress can be caused by a variety factors,
608 physiological (rapid growth rate) and social (overcrowding)
609 ones included (Liles et al., 2015). However, a great deal of
610 individual bird variability in some immunological measures
611 (i.e., stimulation index, heterophils to lymphocyte ratio and
612 lymphocyte blastogenesis) may also be considered (Talebi et
613 al., 1995).

614

615 **Conclusion**

616 In conclusion, the present study suggests that increasing levels
617 of dietary TM meal inclusion in female broiler chickens diets
618 may improve body weight, weight gain and feed intake, but can

619 partially worsen feed efficiency. However, positive effects on
620 carcass traits and haematochemical parameters are observed,
621 along with no negative influence on gut morphology and
622 histological findings. These results confirm previous data
623 concerning the safety of TM utilization in poultry feed, even if
624 legislative issues are still needed to allow insect meal to be
625 used as transformed animal protein to feed monogastric farm
626 animals.

627

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637

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860 **Table 1.** Ingredients (g/kg as fed), apparent metabolizable energy (MJ/kg DM) and nutrient composition (%) of the
861 experimental diets.

Ingredients	First Period (days 1 to 12)				Second period (days 12 to 25)				Third period (day 25 to slaughter)			
	Control	TM5	TM10	TM15	Control	TM5	TM10	TM15	Control	TM5	TM10	TM15
Corn meal	483.2	482.7	488.5	496.6	523.8	535.9	549.3	566.8	566.6	572.2	585.7	605.4
Soybean meal	345.0	333.8	304.0	262.0	317.0	294.0	254.1	203.9	275.5	259.0	219.0	164.0
TM meal	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0
Corn gluten meal	75.5	42.0	23.0	14.5	58.3	24.0	8.0	0.0	56.0	21.0	5.0	0.0
Soybean oil	54.0	50.3	43.5	34.8	64.9	59.9	51.7	41.6	68.9	64.9	56.7	45.8
Dicalcium phosphate	11.0	12.0	13.0	15.5	8.4	9.0	10.5	12.9	7.0	8.0	9.5	12.0
Calcium carbonate	17.5	16.5	16.0	15.0	15.0	15.0	14.5	13.0	14.5	14.0	13.5	12.2
Sodium chloride	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DL-methionine	0.8	0.9	0.9	0.8	0.8	0.9	0.9	0.8	0.4	0.6	0.6	0.5
L-lysine	3.1	1.9	1.2	0.9	2.0	1.3	0.9	0.8	1.3	0.4	0.0	0.0
Threonine	0.1	0.1	0.1	0.1	0.0	0.2	0.3	0.4	0.0	0.1	0.2	0.3
Trace mineral-vitamin premix ¹	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Coline	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
3-phytase	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100	100	100	100	100
AMEn ² (MJ/kg)	12.89	12.89	12.89	12.89	13.28	13.28	13.28	13.28	13.54	13.54	13.54	13.54
Nutrient composition (%)												
DM	86.6	86.6	86.7	86.6	86.7	86.8	86.6	86.8	86.8	86.7	86.7	86.8
CP	23.5	23.5	23.6	23.8	21.3	21.1	21.1	21.1	19.6	19.6	19.6	19.6
EE	7.9	8.3	9.0	9.6	9.0	9.2	9.8	10.3	9.5	9.7	10.4	10.8
NDF	9.4	9.8	10.0	10.1	9.4	9.8	10.0	10.1	9.4	9.7	9.9	10.1
ADF	3.8	4.1	4.3	4.4	3.7	3.9	4.1	4.1	3.5	3.8	3.9	4.0

Nutrient composition (%) ²												
Calcium	1.1	1.1	1.1	1.1	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8
Available phosphorus	0.6	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Digestible methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4
Digestible lysine	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.1
Digestible threonine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8

862 ¹Mineral-vitamin premix (Final B Prisma, IZA SRL), given values are supplied per kg of diet: 2.500.000 IU of vitamin A;

863 1.000.000 IU of vitamin D3; 7.000 IU of vitamin E; 700 mg of vitamin K; 400 mg of vitamin B1; 800 mg of vitamin B2; 400

864 mg of vitamin B6; 4 mg of vitamin B12; 30 mg of biotin; 3.111 mg of Ca pantothenate acid; 100 mg of folic acid; 15.000 mg

865 of vitamin C; 5.600 mg of vitamin B3; 10.500 mg of Zn, 10.920 mg of Fe; 9.960 mg of Mn; 3.850 mg of Cu; 137 mg of I; 70

866 mg of Se.

867 ²Calculated according to INRA 2004 and De Marco et al. (2015).

868 TM, *Tenebrio molitor*; AME, apparent metabolizable energy; DM, dry matter; CP, crude protein; EE, ether extract; NDF,

869 neuter detergent fiber; ADF, acid detergent fiber.

870

871 **Table 2.** Effect of the dietary TM larvae meal inclusion on the growth performance of the female broiler chickens.

Variable ²	Age	Dietary treatments ¹				SEM	P ³	
		C	TM5	TM10	TM15		Linear	Quadratic
LW (g)	12 d	303.15	338.25	339.15	351.83	6.78	0.013	0.352
	25 d	1174.90	1234.77	1183.35	1179.79	14.14	0.775	0.280
	40 d	2078.46	2309.97	2115.91	2084.22	30.51	0.408	0.012
ADG (g)	1-12 d	23.61	26.80	26.89	28.04	0.61	0.012	0.350
	12-25 d	67.06	68.96	64.94	63.69	1.17	0.198	0.512
	25-40 d	60.24	71.68	62.17	60.29	1.91	0.552	0.071
DFI (g)	1-12 d	25.40	28.80	30.80	32.40	0.95	0.006	0.583
	12-25 d	95.00	117.00	109.40	116.60	2.95	0.014	0.129
	25-40 d	184.41	220.23	209.49	225.28	6.85	0.731	0.741
FCR (g/g)	1-12 d	1.08	1.07	1.14	1.15	0.03	0.336	0.925
	12-25 d	1.42	1.74	1.68	1.86	0.05	0.001	0.325
	25-40 d	2.40	2.16	2.13	2.31	0.11	0.775	0.404
	1-40 d	1.78	1.84	1.81	1.95	0.05	0.342	0.730

872 ¹Each mean represents 5 replicates with 8 chicks/replicate (n = 40/treatment).

873 ¹Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level
874 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

875 ²LW, live weight; DFI, daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; n, number of pens.

876 ³Statistical significance: $P < 0.05$.

877

878 **Table 3.** Effect of the dietary TM larvae meal inclusion on the carcass traits of the female broiler chickens.¹

Variable	Dietary treatments ²				SEM	P ³	
	C	TM5	TM10	TM15		Linear	Quadratic
Live weight (LW) (g)	1980	2149	2118	2018	33.68	0.771	0.054
Carcass weight (g)	1377	1501	1500	1276	38.80	0.348	0.025
Carcass weight (% LW)	69.46	69.75	70.77	62.86	1.78	0.256	0.266
Breast (g)	332	388	359	353	10.24	0.688	0.135
Breast (% LW)	16.64	17.92	16.94	17.43	0.24	0.512	0.406
Thigh (g)	411	433	434	402	6.00	0.646	0.026
Thigh (% LW)	20.80	20.20	20.51	20.00	0.16	0.163	0.883
Spleen (g)	2.62	2.84	2.83	2.80	0.13	0.672	0.677
Spleen (% LW)	0.13	0.13	0.13	0.14	0.01	0.742	0.768
Liver (g)	25.01	27.30	27.03	25.68	0.82	0.825	0.302
Liver (% LW)	1.26	1.26	1.27	1.26	0.02	0.921	0.941
Gizzard (g)	36.75	38.20	36.83	34.40	1.03	0.392	0.379
Gizzard (% LW)	1.83	1.77	1.74	1.69	0.04	0.168	0.914
Abdominal fat (g)	40.5	43.3	33.8	43.5	1.29	0.014	0.057
Abdominal fat (% LW)	0.66	0.86	1.08	0.98	0.23	0.005	0.074

879 ¹Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).

880 ²Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level

881 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

882 ³Statistical significance: $P < 0.05$.

883

884 **Table 4.** Effect of the dietary TM larvae meal inclusion on the haematological and serum parameters of the female
885 broiler chickens.¹

Variable ³	Dietary treatments ²				SEM	P ⁴	
	C	TM5	TM10	TM15		Linear	Quadratic
Erythrocyte (10 ⁶ cell/μl)	2.27	2.41	2.47	2.39	0.28	0.073	0.040
Leukocyte (10 ³ cell/μl)	9.28	9.07	9.31	9.70	0.23	0.521	0.562
H/L ratio	0.83	0.68	0.75	0.77	0.03	0.632	0.095
Albumin (g/dl)	1.66	1.35	1.27	1.32	0.06	0.046	0.134
Total protein (g/dl)	3.31	3.80	3.85	4.10	0.14	0.068	0.663
GGT (UI/l)	26.86	22.13	21.61	25.56	1.05	0.629	0.046
AST (UI/l)	189.48	227.18	217.11	211.06	8.62	0.494	0.229
ALT (UI/l)	20.06	21.33	18.57	15.90	1.16	0.164	0.428
Uric Acid (mg/dl)	3.44	4.03	3.28	3.35	0.21	0.617	0.562
Creatinine (mg/dl)	0.36	0.38	0.36	0.38	0.00	0.410	0.957
Triglycerides (mg/dl)	42.44	44.48	52.80	36.03	3.05	0.686	0.133
Cholesterol (mg/dl)	60.33	71.12	70.87	77.80	3.71	0.138	0.799
Glucose (mg/dl)	222.60	221.10	219.70	227.00	1.62	0.431	0.196
Phosphorus (mg/dl)	3.67	4.19	3.93	5.56	0.33	0.067	0.376
Magnesium (mEq/l)	1.30	1.15	1.18	1.15	0.03	0.159	0.380
Iron (μg/dl)	102.44	81.20	81.26	103.89	11.64	0.985	0.392

886 ¹Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).

887 ²Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level
888 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

889 ³H/L, heterophiles to lymphocytes ratio; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase;
890 ALT, alanine aminotransferase; n, number of birds.

891 ⁴Statistical significance: $P < 0.05$.

892

893

894 **Table 5.** Effects of diet, intestinal segment and interaction between diet and intestinal segment on the intestinal
895 morphometric indices of the female broiler chickens.

Index	Fixed effect	d.f. ³	F	P ⁴
Vh (mm)	Diet ¹	3	1.210	0.310
	Intestinal segment ²	2	68.115	< 0.001
	Diet × Intestinal segment	6	0.922	0.483
Cd (mm)	Diet	3	0.891	0.449
	Intestinal segment	2	7.275	0.001
	Diet × Intestinal segment	6	1.593	0.157
Vh/Cd (mm/mm)	Diet	3	0.705	0.551
	Intestinal segment	2	35.195	< 0.001
	Diet × Intestinal segment	6	0.277	0.947

896 ¹Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level
897 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

898 ²Three intestinal segments: duodenum, jejunum and ileum.

899 ³Degrees of freedom.

900 ⁴Statistical significance: P < 0.05.

901 Vh, villus height; Cd, crypt depth; Vh/Cd, villus height to crypt depth ratio.

902 Table 6. Least square means of intestinal morphometric indices in female broiler chickens in
903 relation to diet and intestinal segment.

Index	Fixed effect	Effect levels	Least square mean ¹	SEM
Vh (mm)	Diet ²	C	1.73	0.06
		TM5	1.67	
		TM10	1.57	
		TM15	1.61	
	Intestinal segment ³	DU	2.08 ^a	0.05
		JE	1.65 ^b	
		I	1.20 ^c	
		C	0.20	
Cd (mm)	Diet	TM5	0.20	0.01
		TM10	0.20	
		TM15	0.21	
		DU	0.21 ^a	
	Intestinal segment	JE	0.21 ^a	0.00
		I	0.19 ^b	
		C	8.49	
		TM5	8.51	
Vh/Cd (mm/mm)	Diet	TM10	8.00	0.34
		TM15	8.00	
		DU	10.06 ^a	
		JE	8.13 ^b	
	Intestinal segment	I	6.56 ^c	0.47

904 ¹Means with different superscript letters (a, b) within the same column per fixed effect (i.e.
905 diet, intestinal segment) differ significantly (P < 0.05).

906 ²C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of
907 *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*
908 ³DU = duodenum; JE = jejunum; I = ileum.

909 **Figure legends**

910

911 **Figure 1.** Histological findings of the female broiler chickens. **A)** TM5 group. A normal
912 spleen. 5× Haematoxylin & Eosin stain. **B)** TM5 group. Spleen with severe and diffuse
913 depletion of the white pulp. A high number of apoptotic cells (arrowheads) are observed. 20×
914 Haematoxylin & Eosin stain. **C)** C group. A normal follicle in the bursa of Fabricius. 10×
915 Haematoxylin & Eosin stain. **D)** C group. Bursa of Fabricius with mild and multifocal
916 follicular depletion (arrow) associated with intrafollicular cyst (*). 10× Haematoxylin &
917 Eosin stain.